

## WHAT IS CLAIMED IS:

- 5           1. A substantially pure Type IIG restriction endonuclease obtainable from *Citrobacter* species 2144 (NEB#1398) (ATCC Patent Accession No. PTA-5846) or from *Escherichia coli* NEB#1554 (ATCC Patent Accession No. PTA-5887).
- 10           2. An isolated DNA obtainable from *Escherichia coli* NEB#1554 (ATCC Patent Accession No. PTA-5887) or from *Citrobacter* species 2144 (NEB#1398) (ATCC Patent Accession No. PTA-5846).
- 15           3. Isolated DNA encoding the restriction endonuclease of claim 1, wherein the DNA comprises a first DNA segment expressing an endonuclease and methyl transferase catalytic function and a second DNA segment encoding a sequence specificity function of the restriction endonuclease wherein the first and second DNA segments comprise one or more DNA molecules.
- 20           25           4. A substantially pure restriction endonuclease according to claim 1 capable of recognizing at least one sequence selected from the group consisting of SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34 and SEQ ID NO:35, and cleaving the DNA on both sides of the recognition sequence.

5. A recombinant DNA vector, comprising: at least one of a first DNA segment coding for the restriction and modification domains of CspCI restriction endonuclease and a second segment coding for the specificity domain of the restriction endonuclease.

10 6. A host cell transformed with a first DNA segment coding for the restriction and modification domains of CspCI restriction endonuclease and a second segment coding for the specificity domain of the restriction endonuclease wherein the first DNA segment and the second DNA segment are contained within one or more DNA vectors.

15 7. A method for obtaining the endonuclease of claim 1, comprising cultivating a sample of *Citrobacter* species 2144 (NEB#1398) or a host cell according to claim 6 under conditions favoring the production of the endonuclease; and purifying the endonuclease therefrom.

20 8. A method of making a Type II restriction endonuclease having an altered specificity; comprising:

25 (a) selecting a restriction endonuclease from a set of enzymes wherein each enzyme in the set is characterized by a modular structure having a specificity subunit and a catalytic subunit, the specificity subunit further comprising an N-terminal domain for binding one half site of a bipartite recognition

sequence and a C-terminal domain for binding a second half site of the bipartite recognition sequence;

(b) modifying the specificity subunit; and

(c) obtaining the Type II restriction endonuclease with 5 altered specificity.

9. A method according to claim 8, wherein modifying the specificity subunit in step (b) further comprises substituting the N-terminal domain with a second C-terminal domain or substituting the C-terminal domain with a second N-terminal 10 domain.

10. A method according to claim 8, wherein modifying the specificity subunit further comprises substituting the N-terminal 15 domain or the C-terminal domain or both N-terminal and C-terminal domain with a binding domain from a second restriction endonuclease or methyltransferase.

11. A method according to claim 8, wherein modifying the specificity subunit further comprises mutating the N-terminal 20 domain, the C-terminal domain or both domains to alter the binding specificity.

12. A method according to claim 8, 9, 10 or 11 wherein 25 modifying the specificity subunit further comprises changing the length of the spacer amino acid sequence between the N-terminal and C-terminal domains of the specificity module.

-53-

13. A method according to claim 10, wherein the second restriction endonuclease or methyltransferase is selected from a group consisting of a Type I restriction endonuclease, a Type IIG restriction endonuclease and a  $\gamma$ -type  $m^6A$  methyltransferase.

5

14. A method according to claim 8, wherein the specificity subunit and the catalytic subunit are encoded by different genes.

10